Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2279–2282

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Metal Mediated Protease Inhibition: Design and Synthesis of Inhibitors of the Human Cytomegalovirus (hCMV) Protease

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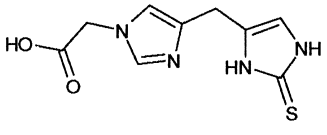
Received 8 June 2000; accepted 1 August 2000

Abstract—A versatile synthetic route to a novel series of bis-imidazolemethanes designed to inhibit the hCMV protease has been developed and a series of potential metal binding inhibitors has been identified. In selectivity assays, the compounds were highly specific for CMV protease and showed no inhibition (IC_{50} >100 μM) of other prototypical serine proteases such as trypsin, elastase, and chymotrypsin. Although the presence of free zinc ions was found to be an absolute requirement for the in vitro biological activity of this class of inhibitor, the potency of the inhibitors could not be improved beyond the micromolar level.

The serine protease of the human cytomegalovirus (hCMV) has become established as an attractive therapeutic target and has been the subject of intense research aimed at discovering potent inhibitors that are effective antiviral agents in vivo.[1] We have recently reported[2] the inhibition of the protease by a series of benzothiopyranone derivatives and other workers[3] have successfully utilized alternative templates to achieve potent inhibition of the enzyme. A number of these compounds have also been shown to be active in models of CMV infection.[4]

The X-ray crystal structure of the protease[5] has revealed a number of unique features noteworthy amongst which is the presence of an active site catalytic triad composed of a serine and two histidine residues. Recently, Katz et al. have reported a conceptually novel approach to the inhibition of serine proteases such as trypsin in which a zinc ion mediated the formation of a ternary inhibitory complex of a low molecular weight metal chelate, metal ion and enzyme.[6] X-ray crystallographic analysis of the complex revealed that the zinc was tetrahedrally coordinated at the enzyme active site and engaged the catalytically important serine and histidine residues of the protease.[7] In view of the presence of an additional zinc ligating histidine residue in the CMV protease active site, we were interested in the application of the metal mediated inhibition concept to the design of novel hCMV protease inhibitors. We report herein the successful realization of this goal and disclose a novel, metal mediated mode of inhibition of hCMV protease.

Examination of the hCMV protease crystal structure suggested that the bis-benzimidazolemethane based metal binding templates (BABIM) could not be readily accommodated within the CMV protease active site. However, we reasoned that excision of the benzo portion of the benzimidazole nucleus would be more successful in generating a geometrically viable ternary complex of enzyme, zinc and inhibitor. In a further design element, we wished to incorporate the ability to display a variety of groups capable of interacting with the protein to allow both for variations in the binding mode of the template and also to allow access to binding pockets on the enzyme. These design requirements could most easily be incorporated into the bis-imidazolemethane (BIM) template **1**.



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We envisaged that alkylation at sulfur of the thioimidazolone followed by amide formation at the carboxylic

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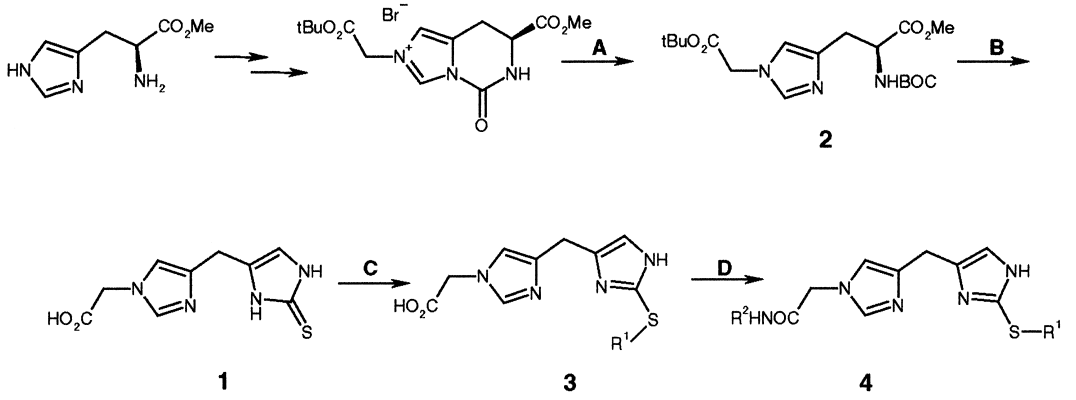
PII: S0960-894X(00)00462-5



Scheme 1. Reagents: (**A**) 1. ^{t}BuOH, ^{i}Pr$_2$EtN; (**B**) 1. TFA, CH_2Cl_2; 2. Na/Hg, NH_4SCN, EtOH/H_2O, 0–5 °C, pH 1.5–3.5; (**C**) 1. R^1X, DMF; 2. CH_2N_2; 3. HCl, Δ; (**D**) R^2NH_2, EDC, HOBt, Et_3N, DMF.

acid could be carried out in a tandem manner and would enable the rapid synthesis of a diverse set of potential metal binding inhibitors of hCMV protease. In addition, we chose to pursue the 4,4′-bisimidazole methane system in preference to the isomeric 2,2′-linked system in order to minimize the potential for oxidation at the linking methylene moiety.

Chemistry

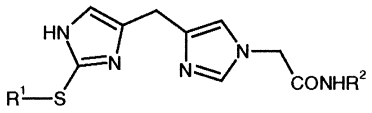
Our approach to **1**, based on that reported by Dreyton and Frew,[8] involved regioselective *N*-alkylation[9] of histidine methyl ester followed by thioimidazolone formation from the resulting pendant α-amino acid (Scheme 1).

Careful monitoring of the reaction pH during the sodium amalgam mediated reduction of the ester **2** was critical in obtaining good conversion to the desired product. However, the need to isolate the highly water soluble **1** from a complex mixture of inorganic byproducts made the overall process inefficient and the isolated yields varied considerably. Fortunately, sulfur alkylation proceeded smoothly with a variety of electrophiles and, on the basis of chemical shift differences in the ^{1}H NMR spectra, the regiochemistry of the thioimidazolone alkylation was readily determined to be at sulfur and not at either of the N1 or N3 alternatives.[10] The resulting crude (thioalkyl)bis-imidazole acids **3** could be coupled with various amines using EDCI as the condensing reagent (Table 1) to give the desired amides **4**.[11]

Having demonstrated the viability of the tandem alkylation–acylation strategy, we next focused on improving the synthetic route with a view to altering the capricious amalgam reduction and thereby allowing for a more facile isolation of the desired product. We considered that the incorporation of a more lipophilic benzyl linker to display the carboxylic acid would allow for both a selective reduction of the amino acid ester in **5** and also considerably improve the overall organic solvent solubility of the template.

These expectations were realized in practice and treatment of **5** with $NaBH_4$/LiCl in methanol:THF[12] and subsequent oxidation of the resulting primary alcohol to the aldehyde followed by thioimidazolone formation proceeded uneventfully to furnish the modified template **6** in good overall yield. Tandem functionalization of **6** using the protocol established above gave access to a second series of potential zinc chelating CMV protease inhibitors (Scheme 2 and Table 2).

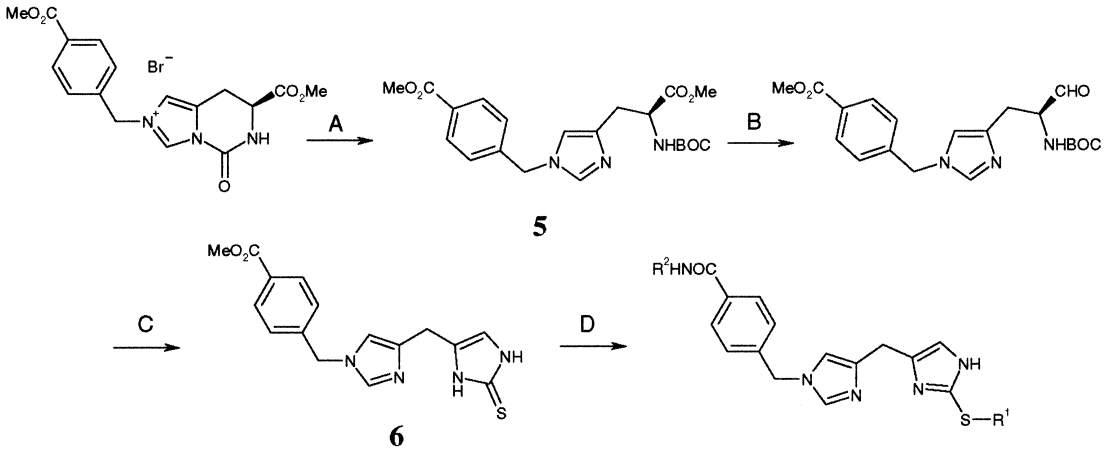
Table 1. A selection of bis(imidazole)methanes **4** prepared according to Scheme 1



Compound	R^1	R^2
7	*n*-Octyl	L-Asp(OBn)$_2$
8	*n*-Octyl	L-Phe-OBn
9	CH_2CO(4-Ph)Ph	*n*-Octyl
10	CH_2-5-Pyrimidinyl	L-Val(OBn)
11	CH_2CN	3,4-(MeO)$_2$CH$_2$Ph
12	CH_2-5-Pyrimidinyl	3,4-(MeO)$_2$CH$_2$Ph

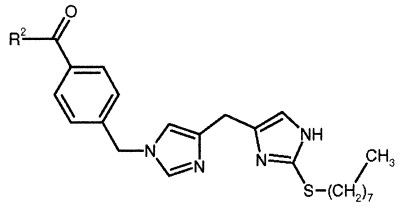
Results and Discussion

The hCMV protease inhibitory activity of the bis-imidazolemethanes was determined[13] by following the increase in fluorescence resulting from the enzymatic processing of a quenched fluorescent peptide substrate. The effect of zinc ion on the potency of the compounds was determined by carrying out the assay with and without 1 mM EDTA. As shown in Table 3 and consistent with the proposed mechanism of action of these compounds, the hCMV protease inhibitory activity was strongly dependent on the presence of free metal ion. In the absence of free zinc, the bis-imidazoles had little or no measurable inhibitory activity towards the protease but upon the addition of 10 μM zinc ion, significant inhibition of the proteolytic activity was observed for some of the compounds, consistent with the formation of a ternary complex of enzyme, zinc, and inhibitor. However, despite the introduction of an extensive range of substituents onto the BIM template, the inhibitory potency of the compounds could not be improved substantially beyond the micromolar level (data not shown).



Scheme 2. Reagents: (**A**) ^{t}BuOH, ^{i}Pr$_2$EtN, Δ; (**B**) 1. $NaBH_4$, LiCl, 2:1 MeOH:THF; 2. $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; (**C**) 1. 4 M HCl/dioxane, 10 °C; 2. NaSCN, H_2O, 100 °C; (**D**) 1. NaOH, MeOH: H_2O, Δ; 2. R^1X, DMF; 3. R^2NH_2, EDC, HOBt, Et_3N, DMF.

Table 2. Bis-(imidazole)methanes prepared according to Scheme 2



Compound	R^2
13	OMe
14	NH $CH_2cC_3H_5$
15	NH CH_2-4-MeOPh
16	NH CH_2CH_2OH

Table 3. hCMV protease inhibition of bis-imidazolemethanes

Compound	hCMV protease IC_{50} (μM)	
	(+) Zinc	(−) Zinc
7	5	15
8	41	125
13	7	>150
14	15	>150

Presumably, the inhibitor template is not involved in making sufficiently extensive contacts with the protein and consequently, the substituents introduced also do not interact optimally with binding site(s) on the enzyme. This may be a consequence of the high affinity of the protease active site for zinc[14] such that additional contacts of the template with the bound zinc are weak and disfavour the formation of a tight, well defined ternary inhibitory complex.

Summary

A versatile synthetic route to a novel series of bis-imidazolemethanes designed to inhibit the hCMV protease has been developed. The potential for rapid, tandem bifunctionalization of the template has been demonstrated and the chemistry used to prepare a range of substituted derivatives. The presence of free zinc ions was found to be an absolute requirement for the in vitro CMV protease inhibitory activity of this class of inhibitor. In selectivity assays, the compounds were generally highly specific for CMV protease and showed no inhibition (IC_{50} >100 μM) of other prototypical serine proteases such as trypsin, elastase and chymotrypsin.[15]

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10. The chemical shift for the protons of the methyl group in 2-(methylthio)imidazole is reported[16] to be 2.52 ppm, whereas in *N*-methyl-2-mercaptoimidazole, the methyl protons resonate at 3.6 ppm.[17] In the ^{1}H NMR of **14**, the corresponding methylene protons resonate at 2.95 ppm consistent with attachment to sulfur.
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